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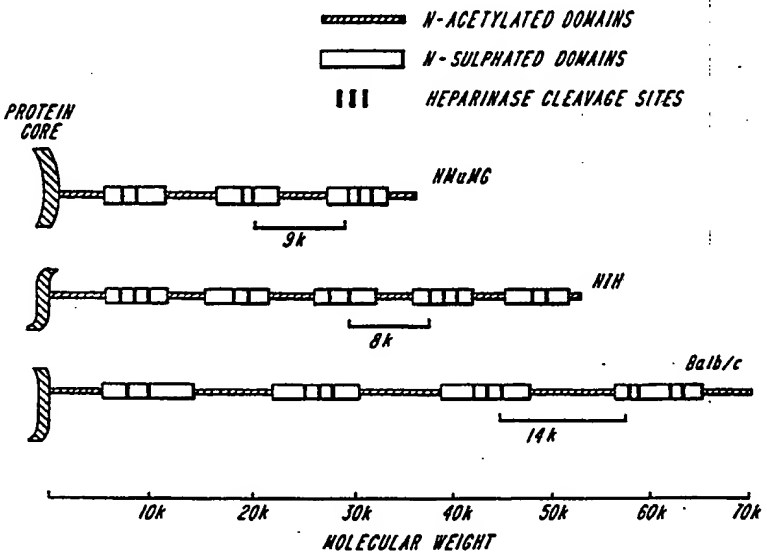

(57) Abstract

It has been discovered that the heparan sulfate chains of proteoglycans vary markedly from one cell type to another and these differences can be exploited for therapeutic and/or diagnostic purposes. In particular, the heparan sulfate chains of cell surface proteoglycans, such as the integral membrane protein, syndecan, isolated from various cells differ not only in size but also in chemical structure (e.g., specific disaccharide composition and distribution). These structural differences appear to be a basis for differences in binding affinity of specific types of cells for particular ligands, and thereby permit the isolation and/or construction of decays, agonists, antagonists and other substrates which can influence or measure biological activity.

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CELL-TYPE SPECIFIC HEPARAN SULFATE PROTEOGLYCANS AND THEIR USES

Background of the Invention

The technical field of this invention is proteoglycan chemistry and, in particular, the use of differentiated proteoglycans and their derivatives for therapeutic and/or diagnostic purposes.

Proteoglycans are commonly found on the surfaces of cells, particularly adherent cells, and bind to wide variety of substances in vivo including, for example, growth factors, enzyme inhibitors, extracellular matrix components and even viruses. In fact, the binding of viruses to cell surface proteoglycans may often be a necessary step in the pathway to viral infection.

For example, the core protein structure of one class of integral membrane cell surface proteoglycans, known as syndecans, is described by one of the present inventors and colleagues in International Patent Application No. PCT/US90/01496, published as W090/12033, and incorporated herein by reference. The core proteins of such proteoglycans typically have molecular weights ranging from about 31 kD to about 35kD and comprise an amino terminus hydrophilic extracellular region, a carboxy terminus hydrophilic cytoplasmic region and a transmembrane hydrophobic region.

The extracellular domains of these cell surface proteoglycans typically have at least one glycosylation site for attachment of a heparan sulfate chain. These chains help define the extracellular domains which serve as attachment sites in vivo. While the structure of the core proteins exhibits considerable diversification, it has been generally assumed that the heparan sulfate chains are largely homologous from one cell type to another.

There exists a need for better diagnostic and therapeutic agents for the study and treatment of diseases and other metabolic conditions, particularly those which appear to be based on binding of bioactive metabolites, pathogens and other factors to cell surface molecules.

Summary of the Invention

It has been discovered that the heparan sulfate chains of proteoglycans vary markedly from one cell type to another and these differences can be exploited for therapeutic and/or diagnostic purposes. In particular, the heparan sulfate chains of cell surface proteoglycans, such as the integral membrane protein, syndecan, isolated from various cells differ not only in size but also in chemical structure (e.g., specific disaccharide composition and distribution). These structural differences appear to be a basis for differences in binding affinity of specific types of cells for particular ligands, and thereby permit the isolation and/or construction of decoys, agonists, antagonists and other substrates which can influence or measure biological activity.

In one aspect of the invention, therapeutic agents can be developed which are isolated, or otherwise derived, from cells which exhibit a high affinity for particular ligand (e.g., a metabolite, pathogen or other factor). Such agents can take the form of soluble proteoglycans having heparan sulfate chains which have been cleaved from selected cells and then purified or, alternatively, synthetic peptides based on native or derivative sequences which have been constructed by genetic engineering techniques. Such soluble agents can be administered to a subject (e.g., a human or animal) in an effective amount to treat a particular disease or metabolic condition, including, for example, promotion of selective wound repair, reduction of tissue-specific inflammation, inhibition of

metastasis, reduction of cholesterol levels in blood, inhibition of viral infections, repair of neuro-muscle junctions, and treatment of leukemia.

In the case of wound repair, one therapeutic approach would be to isolate or construct an agent comprising a soluble heparan sulfate chain derived from a specific cell type which has an affinity for a growth factor, such as the basic fibroblast growth factor, and then administer the agent via a pharmaceutically acceptable carrier to the wound site. The agent would then promote the migration and proliferation of fibroblasts and/or mediate the activities of other repair cells at the wound site.

In another exemplary use, a therapeutic agent comprising a soluble heparan sulfate chain derived from a specific cell type which has an affinity for antithrombins or other circulatory factors can be employed to reduce or prevent arterial plaque deposits by sequestering factors which would otherwise impede the body's ability to eliminate or catabolize cholesterol or other lipoproteins implicated in atherosclerosis.

Likewise, therapeutic agents to treat pathogens can be devised. For example, cells which are naturally vulnerable to herpes simplex infections can be cultured and a soluble heparan sulfate chain with affinity for the herpes virus then derived therefrom. Such a therapeutic agent can be delivered topically or by injection to treat an herpes infection or as a prophylaxis (e.g., during childbirth) against such infections.

The cell-type specific heparan sulfate proteoglycans of the present invention can also be used for diagnostic purposes by employing regents which include heparan sulfate chains having specific affinity for particular ligands as substrates for competitive reactions, in various assays using enzymatic or radiolabeled indicators, according to techniques well known in the art.

The invention will next be described in connection with certain illustrated embodiments; however, it should be clear that those skilled in the art can make various modifications, additions and subtractions without departing from the spirit or scope of the invention.

Brief Description of the Drawings

FIG. 1A is a strong anion exchange (SAX), high pressure liquid chromatograph showing migration over time of heparan sulfate chains isolated from epithelial cells;

FIG. 1B is a SAX high pressure liquid chromatograph showing the composition of heparan sulfate chains isolated from fibroblast cells;

FIG. 1C is a SAX high pressure liquid chromatograph showing the composition of heparan sulfate chains isolated from endothelioid cells;

FIG. 2A is a graph of the elution profile of heparan sulfate chains isolated from epithelial cells and treated with low pH HNO_2;

FIG. 2B is a graph of the elution profile of heparan sulfate chains isolated from fibroblast and treated with low pH HNO_2;

FIG. 2C is a graph of the elution profile of heparan sulfate chains isolated from endothelioid cells and treated with low pH HNO_2;

FIG. 3A is a graph of the elution profile of heparan sulfate chains isolated from endothelial cells and treated with heparitinase;

FIG. 3B is a graph of the elution profile of heparan sulfate chains isolated from fibroblast cells and treated with heparitinase;

FIG. 3C is a graph of the elution profile of heparan sulfate chains isolated from endothelioid cells and treated with heparitinase;

FIG. 4A is a graph of the elution profile of heparan sulfate chains isolated from epithelial cells and treated with heparinase;

FIG. 4B is another graph of the data presented in FIG. 4A but illustrated on a different scale;

FIG. 4C is a graph of the elution profile of heparan sulfate chains isolated from fibroblast cells and treated with heparinase;

FIG. 4D is another graph of the data presented in FIG. 4C but illustrated on a different scale;

FIG. 4E is a graph of the elution profile of heparan sulfate chains isolated from endothelioid cells and treated with heparinase; and

FIG. 4F is another graph of the data presented in FIG. 4D but illustrated on a different scale;

FIG. 5 is a schematic illustration of the differences in size and chemical structure of heparan sulfate chains isolated from various cell types.

Detailed Description

The heparan sulfate chains of cell surface proteoglycans typically contain approximately equal amount of N-acetylated and N-sulfated disaccharides, which are arranged in a mainly aggregated manner into distinct structural domains. However, it has been found that the molecular fine structure (particularly, O-sulfation) varies markedly between different cell types and between proteoglycans.

In the experimental studies reported below, variations were defined by studying the structure of heparan sulfate chains on a particular class of integral membrane proteoglycans, known as syndecans, derived from three distinct cell types: simple epithelial (NMuMG mammary cells), fibroblasts (NIH 3T3 cells) and endothelioid cells (Balb/c 3T3 cells).

In each instance, the syndecan was affinity purified from the conditioned medium of cell cultures using a monoclonal antibody against the syndecan core protein. For a further discussion of the structure and nature of the syndecan core protein, see the above-referenced International Patent Application No. PCT/US90/01496 and the various scientific articles cited therein, which are hereby incorporated by reference.

Since the molecular cloning of the syndecan core protein from mouse mammary epithelia (Saunders et al. 1989 J. Cell Biol. 108: 1547), cDNA-derived amino acid sequences have become available for other PG core proteins that are sufficiently similar to indicate common ancestry. These proteins which

constitute the syndecan family have a similar domain structure, highly conserved sequences, and a conserved exon organization in the genes studied to date. The syndecan-1 gene has been shown to map to human chromosome 2p23 (Ala-Kapee et al. 1990 Somatic Cell Molec. Genet. 16: 501) and to the syntenic region in the mouse on chromosome 12 (Oettinger et al. 1991 Genomics 11: 334), while the syndecan-2 gene maps to human chromosome 8q23 (Marynen et al. 1989 JBC 264: 7017).

Where studied, the core proteins of the syndecan family have similar chemical properties. Each is a heparan sulfate containing proteoglycan, and may also contain chondroitin sulfate.

Evolution of the syndecans from a common ancestor appears to have maintained the location and nature of the putative glycasaminoglycan (GAG) attachment sites, the protease susceptible site adjacent to the plasma membrane, and the transmembrane and cytoplasmic domains. Size, GAG attachment sites, and sequences indicate a closer structural relationship between the proteins. In a number of syndecan genes, the N-terminal GAG attachment region is encoded by a separate small exon (Hinkes et al. 1991 J. Cell Biol. 115: 125a). Additional GAG attachment sites typically reside near the plasma membrane and are syndecan-type in sequence. These sites are not uniformly substituted with heparan sulfate or chondroitin sulfate on syndecan-1 from mouse mammary epithelial (NMuMG) cells.

The regions C-terminal to the conserved putative protease-susceptible site are most highly conserved. A single exon in the mouse syndecan-1 gene and in the rat and chick syndecan-3 genes encodes the identical portion of this region. The tyrosine that completes the transmembrane domain and the three tyrosines in the cytoplasmic domain are invariant. The length and sequence between the transmembrane domain and the first tyrosine are conserved and could account, in syndecan-1 and -3, for a tyrosine internalization signal. However, the distance between the next tyrosine differs, possibly providing individual syndecans with specificity towards interacting proteins. One of the tyrosines fits a consensus sequence for tyrosine phosphorylation.

Syndecan-1 isolated from several sources is a hybrid proteoglycan, containing both chondroitin sulfate and heparan sulfate. These chains are known to be linked via a xyloside to serine residues in proteins (Roden, L., The Biochemistry of Glycoproteins and Proteoglycans (1980) 267-371; and Dorfman, A., Cell Biology of Extracellular Matrix (1981) 119-138). The synthesis of both types of chains is initiated by a xylosyltransferase that resides in either the endoplasmic reticulum or the Golgi, (see Farquhar, M.G., Ann. Rev. Cell Biol. (1985) 1:447-488) and by three Golgi-localized gylcosyltransferases (Geetha-Mabib, et al. 1984 J. Biol. Chem. 259:7300-7310). Specific chain elongation subsequently involves the sequential action of an N-acetylgalactosaminyltransferase and a glucuronosyltransferase for chondroitin sulfate, and

an N-acetylglucosaminyltransferase and a glucuronosyltransferase for heparan sulfate. This specific chain elongation must involve recognition of unique structural features of the core protein, indicating that distinct peptide sequences exist at chondroitin sulfate and heparan sulfate attachment sites.

It is considered to be within the scope of this invention that cell-type specific hepara sulfate chains can be derived from naturally occuring or recombinant syndecans, or fragments thereof.

Disaccharide composition was analyzed by depolymerization with polysaccharide lyases and strong anion exchange (SAX) HPLC of disaccharide products. Radiolabeled disaccharide were detected using an in-line radioactivity monitor (Canberra Packard Flo-one A-250).

The sizes of intact chains and large oligosaccharides were estimated by Sepharose CL-6S chromatography (1x120 cm, 500 mM NH_4 HCO_3 1 4ml/hr).

Initial oligosaccharide mapping was carried out by gel filtration on Bio-Gel p6 columns (1x120cm, 500mM NH. HCO_3, 4ml/hr) after treatment with low pH HNC_2, heparitinase or heparinase.

In FIG. 1A-1C, the disaccharide composition of the three heparan sulfate species was analyzed by SAX HPLC. The results of this analysis are also summarized in Table 1 below, and compared to data from skin fibroblast heparan sulfates.

TABLE 1

DISACCHARIDE COMPOSITION

The data below summarizes the disaccharide composition of the different syndecan HS species. For comparison, data from skin fibroblast HS is also shown.

Standard No.	Disaccharide Structure	Human skin Fibroblast HS	Syndecan HS NMuMG	Syndecan HS NIH	Syndecan HS Balb/c
1	UA-GlcNAc	46.0	44.3	46.1	46.2
2	UA-GLcNAc(HS)	5.4	5.5	6.0	4.7
7	UA(2S)-GlcNAc	1.1	2.6	2.5	1.8
3	UA-GlcNSO$_3$	27.7	25.0	25.3	28.2
4	UA-GlcNSO$_3$(6S)	2.4	3.2	3.6	2.1
5	UA(2S)-GlcNSO$_3$	15.4	11.9	5.7	9.8
6	UA(2S)-GlcNSO$_3$(6S)	2.0	7.5	10.8	7.2
Sulphates / 100 di		75.8	85.8	84.8	80.1
O-sulphates / 100 di		28.3	38.2	39.4	32.8
N-sulphates / 100 di		47.5	47.6	45.4	47.3
N/O sulphate ratio		1.68	1.25	1.13	1.44

As can be seen from FIG. 1 and Table 1, each heparan sulfate species displays a unique disaccharide profile, the most obvious variation being the level of highly sulfated disaccharides: UA(2S)-GlcNSO$_3$ and UA(2S)-GlcNSO$_3$(6S). All of the three species show characteristic levels of N-sulfation (approximately 45-48%). In contrast, their O-sulfate content (and N/O sulfate ratio) varied markedly. In addition, all three heparan sulfate species derived from the cell surface proteoglycan, syndecan, were more highly O-sulfated than the fibroblast heparan sulfate, which is likely a mixture of heparan sulfate species.

In FIGS. 2A-2C, the domain structure of the heparan sulfate chain derived from various cell types was analyzed by Bio Gel P6 oligosaccharide mapping after treatment with low pH base HNO_2. In FIG. 3A-3C, similar mapping was obtained for each of the heparan sulfate chains derived from the different cell types after treatment with heparitinase. In FIGS. 4A-4F, the mapping was obtained after treatment with heparinase.

Based on the P6 mapping data, the distribution of specific linkage types (i.e., contiguous, alternating or spaced apart), as summarized in Table 2, below.

TABLE 2

DISTRIBUTION OF DISACCHARIDES

The data below summarizes the distribution of specific disaccharide types. It is based on calculations from Bio-Gel P6 mapping profiles generated with the specific cleavage reagents shown.

		NMuMG	NIH	Balb/c
N-sulphated disaccharides (HNO_2-susceptible)		50.0	48.4	47.9
Distribution*	C	55	52	45
	A	25	36	33
	S	20	12	22
GlcA-containing disaccharides (heparitinase-susceptible)		61.0	68.7	74.3
	C	76	81	84
	A	8	6	7
	S	16	13	9
IdoA(2S)-containing disaccharides (heparinase-susceptible)		15.9	12.0	16.4
	C	38	42	56
	A	19	13	14
	S	43	45	30

* Distribution:

C = proportion of linkage in contiguous sequences
A = proportion in alternating sequence with a resistant linkage
S = proportion spaced apart by the two or more resistant linkages

The size of the intact chains and large heparitinase-resistant oligosaccharides was estimated by sepharose CL-6S chromatography, as shown below in Table 3.

TABLE 3

SIZE OF HS CHAINS AND HEPARINASE-RESISTANT DOMAINS

	NMuMG	NIH	Balb/c
Intact chain size (kDa)	35	52	75
Average heparinase-resistent domain size* (kDa)	9	8	14
(Approximate size range)	(7-15)	(6-14)	(11-19)

*These domains are the large heparinase-resistant oligosaccharides obtained in the Vo from Bio-Cel P6 profiles (Fig. 4)

As can be seen above, the P6 mapping profiles (shown in Table 2) indicate significant differences in the content and distribution of GlcA residues (heparitinase susceptible) and IdoA(2S) residues (heparinase susceptible). The mapping profiles for N-sulfated disaccharides (as shown in FIGS. 2A-2C) were broadly similar in characteristics of cell-derived heparan sulfate. Nonetheless, the three species of heparan sulfate chains varied markedly in size (as shown in Table 3). The average spacing of heparitinase cleavage sites (clustered within N-sulfated domains) also differed between the heparan sulfate species (Table 3).

In FIG. 5, schematic diagrams of the domain structure of the three heparan sulfate species described above are presented. These schematic structures reflect the spacing of N-sulfated domains containing clusters of heparitinase-susceptible disaccharides (average size of spacings as indicated). They represent a simplified picture since variations in the precise position in spacing of the sulfated domains occurs (see Table 3), and the N-sulfate groups are sparsely distributed in the N-acetylated domains.

Based on the foregoing, it should be clear that the identification of specific heparan sulfate chains can be readily derived from the cell surface proteoglycans of different cell types, particularly from syndecans, and that such cell-type specific heparan sulfate chains or protions thereof can be used for various therapeutic and diagnostic purposes.

The therapeutic agents of the present invention can be administered topically, orally, or by intravenous, intramuscular, or subcutaneous routes.

Topical preparations can be prepared by mixing the active compounds with a suitable emollient, lubricant, or oil, such as glycerol, petrolatum, or mineral oils. Surfactants and other agents can be added to ensure dispersion and/or increase the shelf-life of the preparation. The active compounds may be orally administered, for example, with an inert dilutent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions syrups, wafers, and the like.

For both topical and oral preparations, such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of the unit. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: A binder,

as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dialcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compounds sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparations and formulations.

The active compounds may also be administered vaginally, parenterally or intraperitoneally. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacterial and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases., it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monosterate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required,

followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

What We claim is:

Claims

1. A composition for binding a biological ligand comprising a heparan sulfate chain derived from a native proteoglycan of a cell exhibiting a cell-type specific binding affinity for said ligand, said heparan sulfate chain having a pattern of sulfation which affects the cell-type specific binding affinity for the ligand.

2. The composition of claim 1 wherein the proteoglycan is an integral membrane proteoglycan.

3. The composition of claim 2 wherein the membrane proteoglycan is a syndecan.

4. The composition of claim 1 wherein the pattern of sulfation which provides the cell-type specific affinity for the ligand comprises a cell-type specific degree of O-sulfation of the heparan sulfate.

5. The composition of claim 1 wherein the pattern of sulfation which provides the cell-type specific affinity for the ligand comprises a cell-type specific clustering of N-sulfated domains in the heparan sulfate chain.

6. The composition of claim 1 wherein the heparan sulfate chain has a pattern of uronic acids which affects the cell-type specific binding affinity for the ligand.

7. A pharmaceutical preparation comprising a therapeutic agent for binding a biological factor, the therapeutic agent comprising a heparan sulfate chain having an affinity for said factor and in an amount effective to modify the level of free factor in a host, and a pharmaceutically acceptable carrier, said heparan sulfate chain having a pattern of sulfation which provides a cell-type specific binding affinity for the ligand.

8. The preparation of claim 7 wherein the therapeutic agent is derived from an integral membrane proteoglycan.

9. The preparation of claim 8 wherein the membrane proteoglycan is a syndecan.

10. A method of sequestering an undesirable biological factor in a subject, the method comprising administering a therapeutic agent to the subject comprising a heparan sulfate chain having an affinity for said factor and in an amount effective to modify extracellular levels of said factor, said heparan sulfate chain having a pattern of sulfation which provides a cell-type specific binding affinity for the factor.

11. The method of claim 10 wherein the therapeutic agent is derived from an integral membrane proteoglycan.

12. The method of claim 11 wherein the membrane proteoglycan is derived from a syndecan.

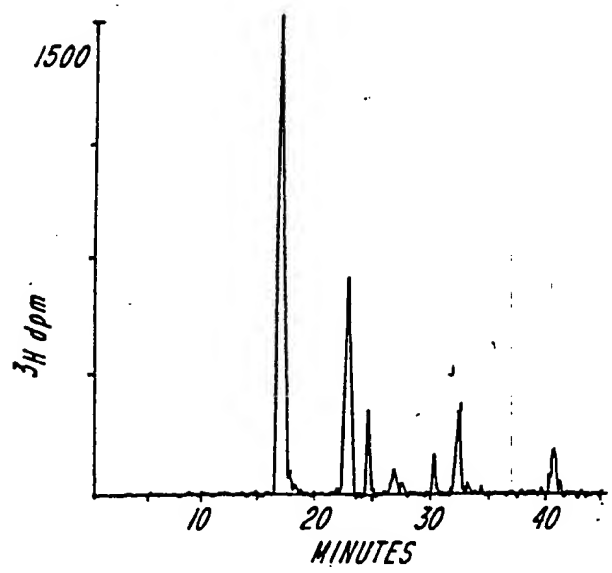

FIG. 1A

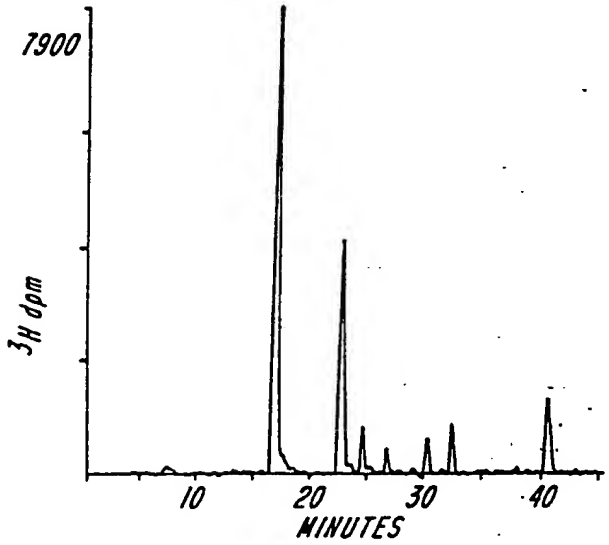

FIG. 1B

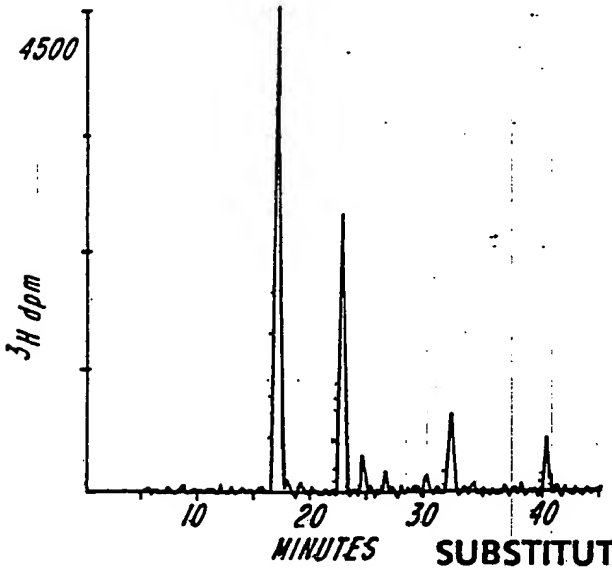

FIG. 1C

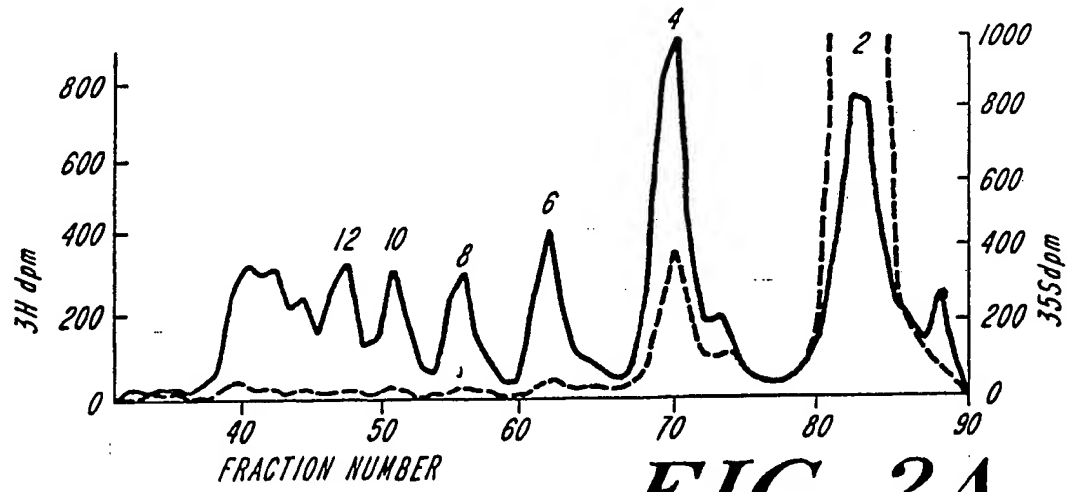

FIG. 2A

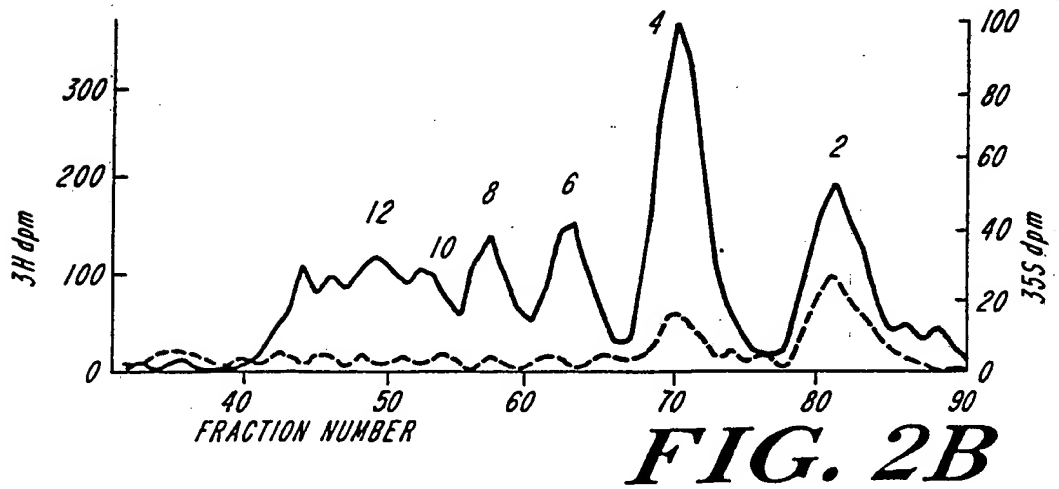

FIG. 2B

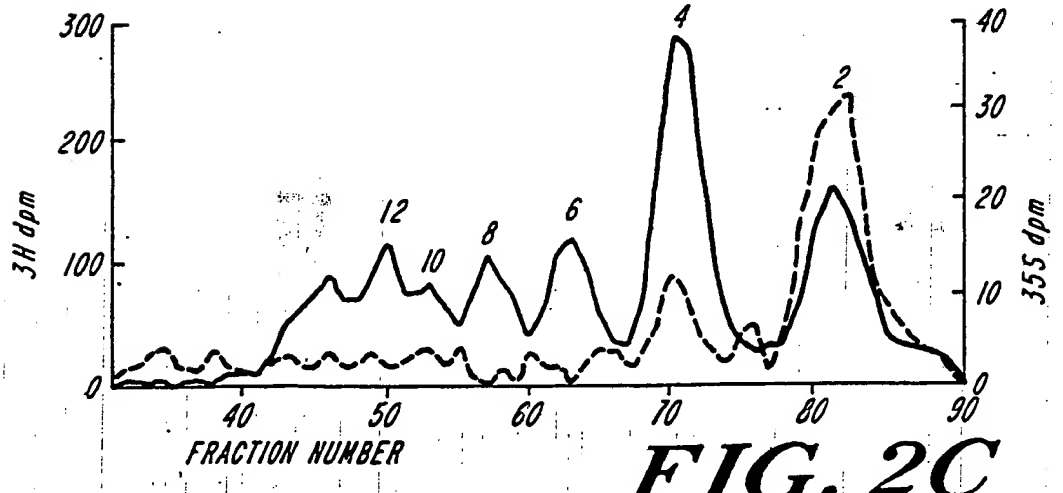

FIG. 2C

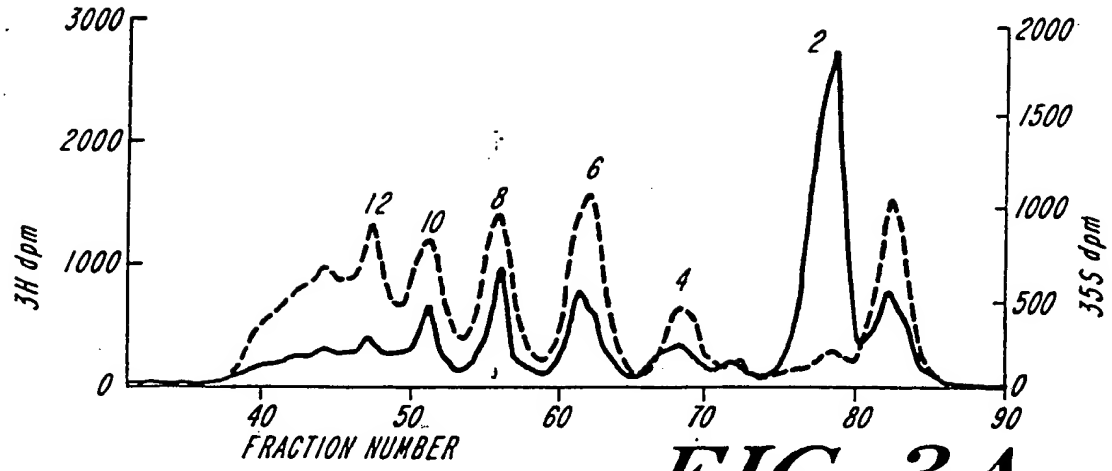

FIG. 3A

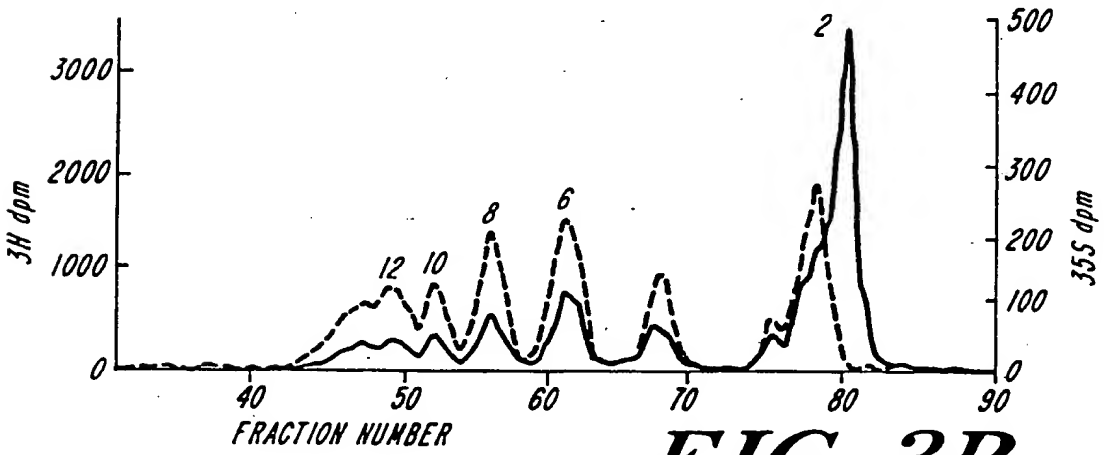

FIG. 3B

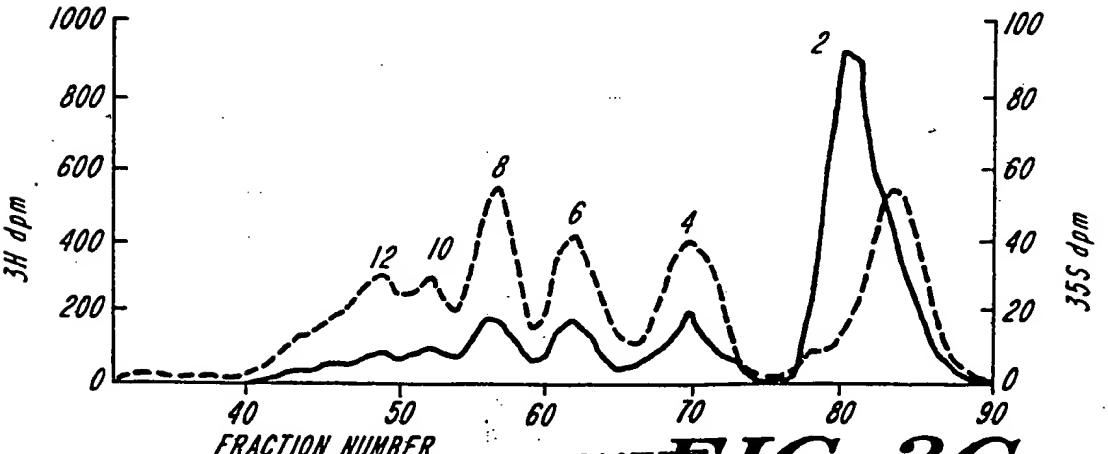

FIG. 3C

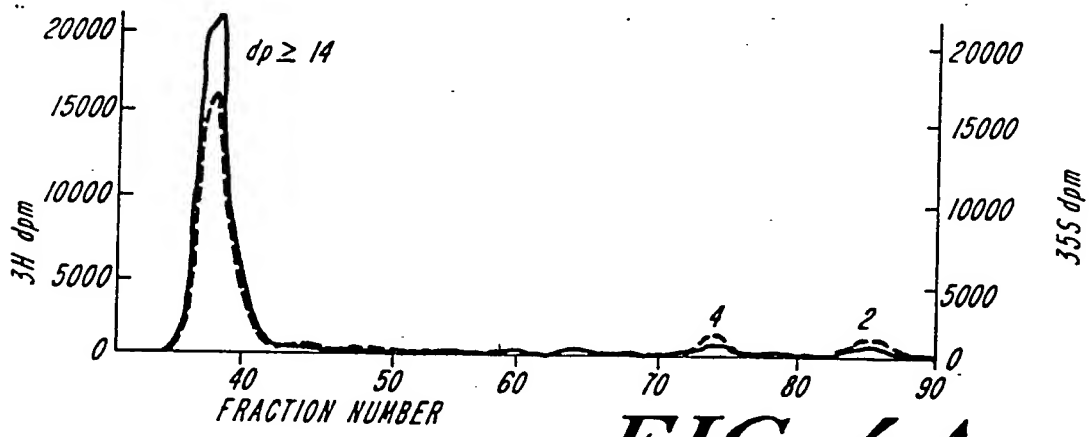

FIG. 4A

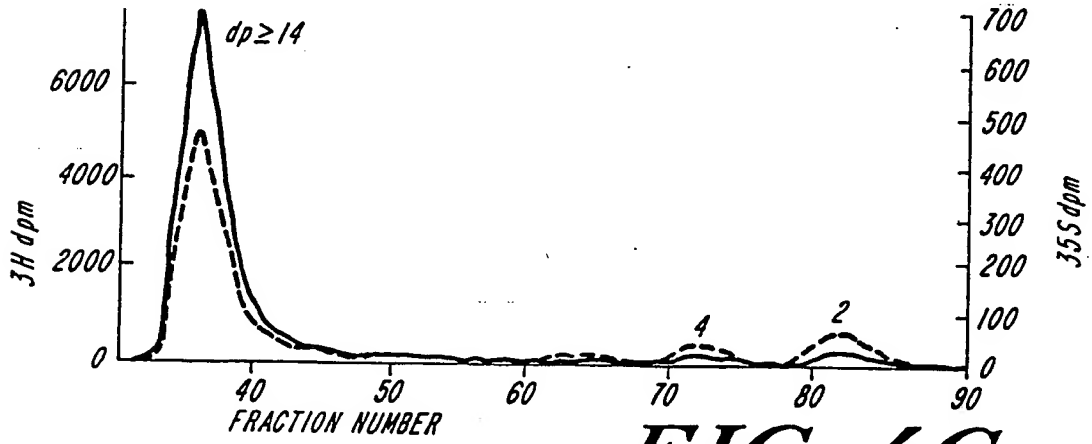

FIG. 4C

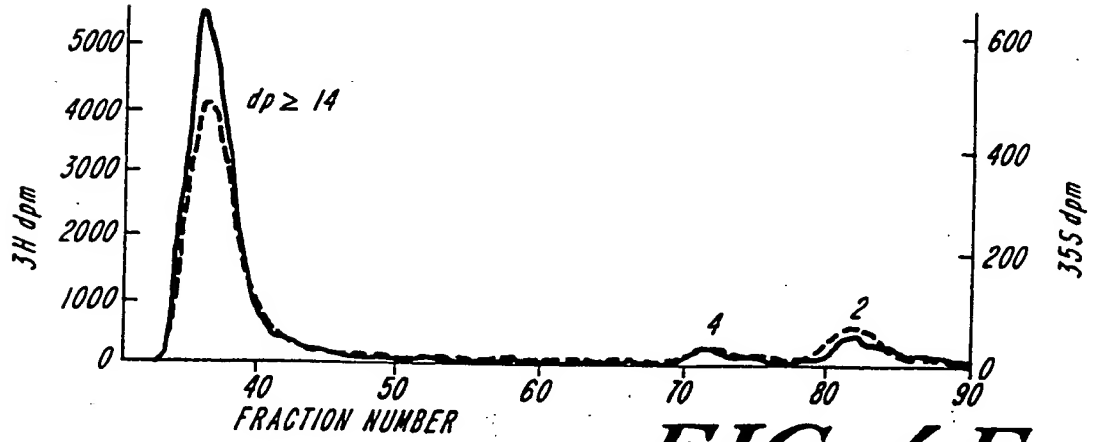

FIG. 4E

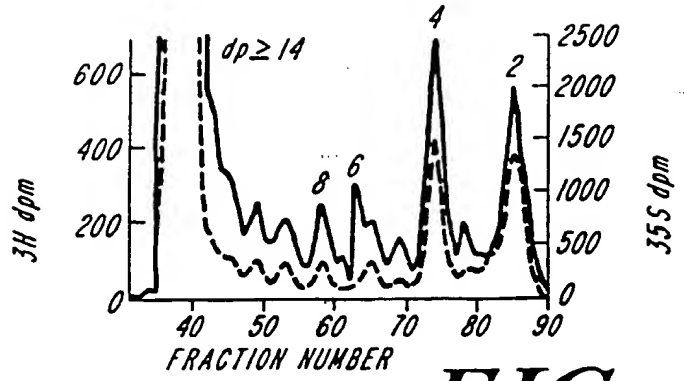

FIG. 4B

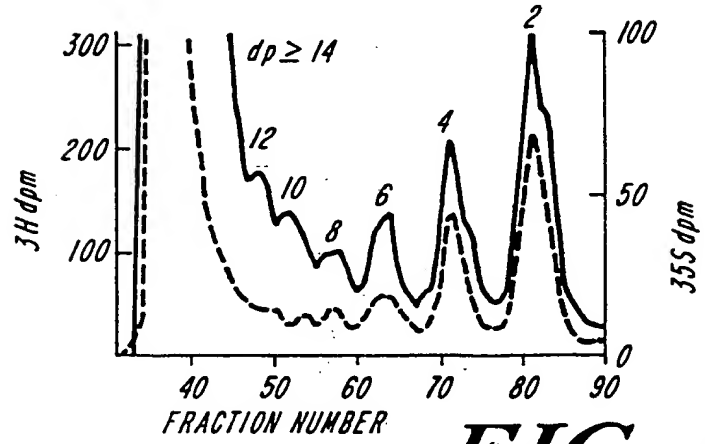

FIG. 4D

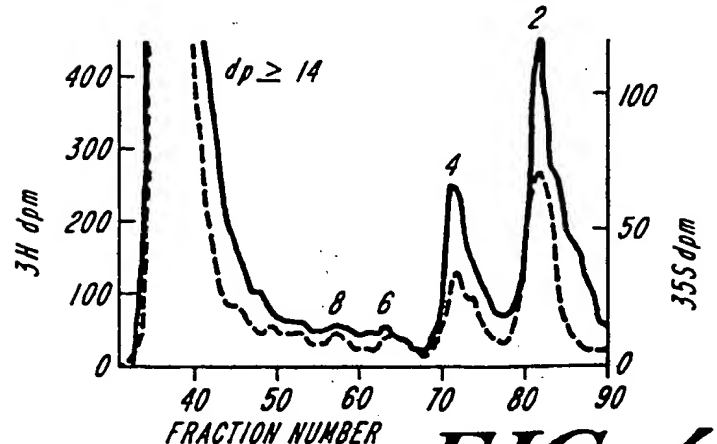

FIG. 4F

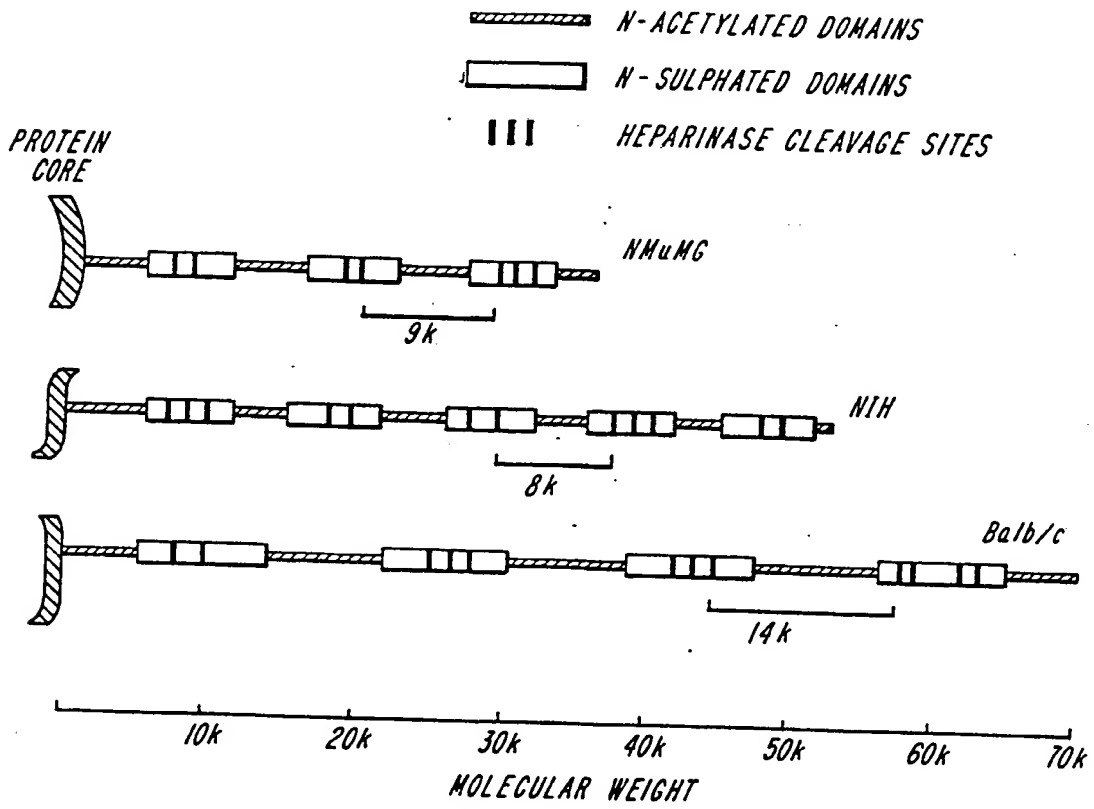
N-ACETYLATED DOMAINS
N-SULPHATED DOMAINS
HEPARINASE CLEAVAGE SITES
PROTEIN CORE
NMuMG
9k
NIH
8k
Balb/c
14k
10k
20k
30k
40k
50k
60k
70k
MOLECULAR WEIGHT

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/07495

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12P19/26; A61K31/73; C08B37/10

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K ; C12P ; C08B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO,A,9 007 712 (BISSENDORF PEPTIDE GMBH) 12 July 1990 cited in the application see page 4, line 7 - line 10 see page 42, line 30 - page 44, line 9 ---	1-3,7-12
A	EP,A,0 341 006 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 8 November 1989 see page 2, line 37 - line 47 see page 3, line 31 - line 45 see page 5, line 8 - line 24 -----	1,2,7,8, 10,11

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 NOVEMBER 1992	27. 11. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MONTERO LOPEZ B.

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/US 92/ 07495

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-12 are directed to a method of treatment of the human/animal body (Rule 39.1(iv)) the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9207495 SA 64420

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 19/11/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9007712	12-07-90	None	
EP-A-0341006	08-11-89	US-A- 4945086	31-07-90
		JP-A- 2084402	26-03-90